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INFLUENCE OF GARLIC EXTRACT ON ANTIOXIDANT STATUS OF CHICKEN

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ABSTRACT

In 2006 the European Union banned the feeding of antibiotic growth promoters because of possible risk of drug resistance in human pathogens bacteria. This is the reason for the study of various phytogenic additives and their extracts as a natural source of biologically important compounds. Antimicrobial substances are a commonly included in chicken feed rations. They are used mainly as prevention against various diseases, and also to stimulate growth. The beneficial effects of garlic on animal organism resulting from their antimicrobial, antioxidative and antihypertensive properities. Studies focused on growth, conversion and meat quality of different types of animals indicate its positive effects. In our experiment we studied the influence of garlic extract in a dose of 0, 10 g and 15 g per 1 kg of chicken feed mixture. We focused on weight gains and antioxidant status of an organism. The experiment took 39 days. 54 seven-day-old chickens were included in the experiment. The chickens were weighed once a week, when aged 11, 17, 24, 31 and 38 days, at the same time of the day. The chickens had ad libitum access to feed ration and water. The chickens were taken blood sample at the end of the experiment when 39 days old. Their antioxidant status were measured using ABTS, FRAP and DPPH methods. Our results show that owing to higher concentration of garlic extract in feed ration the antioxidant status of observed chickens was increased. DPPH method showed an increase in antioxidant status of both experimental groups by 38% (a group with a dose of 10 g/kg of mixture) and by 46% (a group with a dose of 15 g/kg of mixture) compared to the control group. When using FRAP method, antioxidant status of both G10 and G15 groups increased by 24%, resp. 16%. No evidential differences in antioxidant activity between the experimental groups and control group were found using ABTS method. The supplement of garlic extract into a feed ration did not have any influence on weight gains of chickens.

Keywords: poultry; garlic extract; antioxidant status

INTRODUCTION

Garlic is one of the earliest plants ever cultivated **(Nevrkla et al., 2013, Prasad et al., 2009)**. It has been known for thousands of years in folk medicine of Greeks and Egyptians **(Horton et al., 1991, Togashi et al., 2008)**. It is known as spice and herbal medicine for treatment as well as prevention against various diseases **(Ashayerizadeh et al., 2009, Khan et al., 2012)**. In body it has various effects such as aggregation of platelets, decrease in arterial blood pressure or prevention against fatty infiltration of liver. Both *in vivo* and *in vitro* studies proved that matured garlic extract stimulates functions of immune system **(Prasad et al., 2009)**.

Garlic contains at least 33 substances containing sulphur, enzymes and amino acids, minerals including selenium. The main active components in garlic are allicin, ajoene, dialkyl polysulfides, s-allylcysteine (SAC), diallylsulfide, S–methyl–cystein sulfoxide and s–allylcysteine sulfoxide which may be responsible for healing effect of garlic **(Togashi et al., 2008)**. Chemistry of garlic is a complex mechanism, which has probably evolved as an individual protective mechanism against microorganisms and other impairment. Whole garlic typically contains 1% of alliin, together with (1)-S-methyl-L-cysteine sulfoxide (methiin) and (1)-S-(trans-1-propenyl)-L-cysteine sulfoxide. S-(2-carboxypropyl) glutathione, g-glutamyl-S-allyl-L-cysteine, g-glutamyl-S-(trans-1-propenyl)-L-cysteine and g-glutamyl-S-allyl-mercapto-L-cysteine are also present in cloves of garlic. Allicin naturally cumulates if garlic is stored in lower temperatures. A garlic bulb contains in average 0.9% of G-glutamylcysteine and 1.8% of alliin. Apart from these main sulphur compounds an unimpaired garlic bulb may also contain a small amount of SAC, but no allicin. SAC is created during catabolism from g-glutamyl cysteine. Typical volatile substances in milled garlic and garlic volatile oils are diallyl sulfid (DAS), diallyl disulfid (DADS), diallyl trisulfide methyl allyl disulfide, methyl allyl trisulfid, 2-vinyl-1,3-dithiin, 3-vinyl-1,2-dithiin a E, Z-ajoene **(Amagase et al., 2001)**. Allicin, a major product of garlic aroma, is known as an antimicrobial substance, which is created from alliin. Enzyme alliinase, and corresponding cystein sulfoxid aliin, can be found in different parts of garlic plant. If a cell is impaired, alliinase lyses alliin into allicin, pyruvate and ammonium.

Considering that garlic contains a high concentration of selenium, an important part of antioxidant system, higher antioxidant potential can be expected **(Horky, 2014a, Horky, 2014b, Horky et al., 2012a, Horky et al., 2013)**. It is not only selenium that takes place in antioxidant

system, but also other substances present in garlic **(Horky et al., 2012b, Jancikova et al., 2012)**. There are several physiological processes in microorganisms which are influenced by allicin, such as biosynthesis of lipids, RNA synthesis or decreasing level of lipids and aggregation of platelets in mammals **(Focke et al., 1990)**. Allicin functions as an antibiotics destroying sulfhydryl group of enzymes, inhibits fermentation and stimulates gastric secretion, which leads to prophylactic precaution against bacterial infections of gastrointestinal tract. In the last decade, garlic has been added into feed doses of poultry due to its influence on gains **(Khan et al., 2012, Togashi et al., 2008)**. Its effect is stronger during first weeks of life of birds **(Togashi et al., 2008)**.

The aim of our study is to find out an influence of garlic extract on weight gains, feed consumption, and weight of carcass bodies and antioxidant status of broiler chickens.

MATERIAL AND METHODOLOGY

Experiment design

54 seven-day-old male chickens of Ross 308 type were included in the experiment. Chickens were divided into 3 groups. One group was a control one, other two groups were fed with a feed ration with added garlic extract of concentration 10 and 15 g per 1 kg of feed mixture.

Animals and their conditions

Chickens were weighed, marked with wing stamps and then divided into groups and put into balancing cages. They were divided into weight-matched groups before the experiment started. Following weightings were done at age of 11, 17, 24, 31 and 38 days, at the same time of the day.

Animals could access water and feed ration *ad libitum*. Feed ration was mixed up from these components: wheat 25%, corn 37%, soya extract grain 28%, sunflower oil 6%, mixture of vitamins and minerals without anticoccidials 3%, monocalciumphosphate 0.8%, grounded calcite 0.2%. Garlic extract of concentration of 10 and 15 g per 1 kg of feed ration was added into feed mixture.

Consumption of feed ration was recorded for each group.

Light regime was set to 6 hours of darkness and 18 hours of light. At the age of seven days temperature was set to 29.9 °C (with relative humidity of 50%). Temperature was being lowered every day by 1 °C to a level of 23 °C.

Sample preparation

The experiment was ended when chickens reached aged of 39 days. Blood was taken from jugular vein into heparin test-tube when chickens were killed by decapitation.

Determination of antioxidant activity and total proteins

Spectrophotometric measurements of antioxidant activity and total proteins were carried out using the BS-400 automated chemical analyser (Mindray, Shenzhencity, China). Transfer of samples and reagents was provided by a robotic arm equipped with a dosing needle (error of dosage not exceeding ±5% of volume). Cuvette contents were mixed immediately after addition of reagents or samples by an automatic mixer including a stirrer.

Determination of total proteins by the Biuret method

The Biuret method is a test used for detecting the presence of peptide bonds. In the presence of peptides, a copper (Hysing & Wiik) ion forms a violet-coloured complex in an alkaline solution.

A 150 µL volume of Biuret reagent (100 mM potassium sodium tartrate, 100 mM sodium hydroxide, 15 mM potassium iodide and 6 mM copper$^{(II)}$ sulfate) is pipetted into a plastic cuvette with subsequent addition of 3 µL of sample. Absorbance is measured at λ = 546 nm after 10 minutes of incubation. Resulting value is calculated from the absorbance value of the pure Biuret reagent and from the absorbance value after 10 minutes of incubation with the sample.

Determination of antioxidant activity by the ABTS test

The procedure for the determination was taken from a publication by Sochor *et al*. **(Sochor et al., 2010a)**. A 150 µL volume of reagent. Seven mM 2,2´-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS$^{\bullet}$) and 4.95 mM potassium peroxodisulphate was mixed with 3 µL of the sample. Absorbance was measured at 660 nm for 10 minutes.

Determination of antioxidant activity by the FRAP method

The procedure for this determination was taken from a paper by Sochor *et al*. (Sochor et al., 2010b). A 150 µL volume of reagent was injected into a plastic cuvette with subsequent addition of a 3 µL sample. Absorbance was measured at 605 nm for 10 minutes.

Determination of antioxidant activity by the DPPH test

This procedure for the determination was taken from publications by Sochor *et al*. **(Sochor et al., 2010a)**. A 150 µL volume of reagent (0.095 mM 2,2-diphenyl-1-picrylhydrazyl - DPPH$^{\bullet}$) was incubated with 15 µL of the sample. Absorbance was measured at 505 nm for 10 minutes.

RESULTS AND DISCUSSION

When evaluating antioxidant status, antioxidant activity was evaluated using DPPH test, methods FRAP and ABTS. Resulting values of antioxidant activities were converted to 1 gram of protein. According to anticipated hypothesis, in groups with addition of garlic extract we found a direct influence on above-mentioned markers of antioxidant potential of organism.

Evaluation of antioxidant activity

Antioxidant activity is a marker of total amount of antioxidants in a given sample. It is a value, which is used to evaluate the ability of organism to uptake free radicals, protect against their creation or to change them into less reactive forms. Lowered antioxidant activity leads to oxidative stress, which is related to higher rate of impairment of organism (disease, impaired productive and reproductive performance).

When evaluating antioxidant activity using DPPH test (Figure 1) was in both experimental groups observed an increase in antioxidant activity determined by DPPH test by 38% (G10) and 46% (G15) compared to control group.

When evaluating antioxidant activity using FRAP method (Figure 2), increase in antioxidant activity was found in both groups, in G10 by 24% and in G15 by 16%. These values, however, were not statistically significant.

Third method used to evaluate antioxidant activity was ABTS method (Figure 3). No relevant differences between control and experiment groups were found using this antioxidant marker.

Evaluation of weight of chickens

General health, influenced by antioxidant status of an organism, directly affects also efficacy of domestic animals. In our experiment the gains of observed animals were evaluated in time intervals. On days 11, 17 and 24 of the experiment weights of control group animals and G10 and G15 animals did not change considerably. From day 31 weight gains were observed in control group compared to G10 (by 6%) and G15 (by 9%). The same trend was

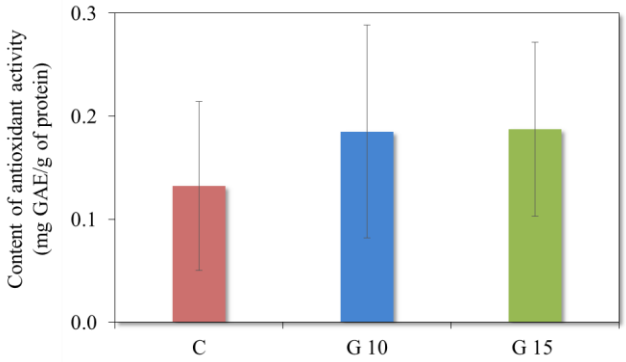

Figure 1 Evaluation of antioxidant activity using DPPH method (C - concentration 0% of garlic extract, G 10 - 10 g/kg of feed ration, G 15 - 15 g/kg of feed ration).

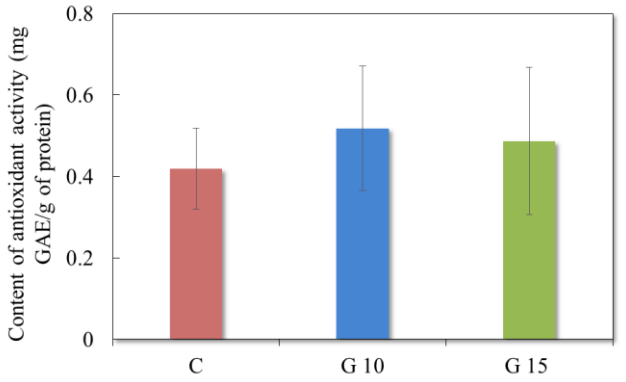

Figure 2 Evaluation of antioxidant activity using FRAP method (C - concentration 0% garlic extract, G 10 - 10 g/kg of feed mixture, G 15 - 15 g/kg of feed mixture).

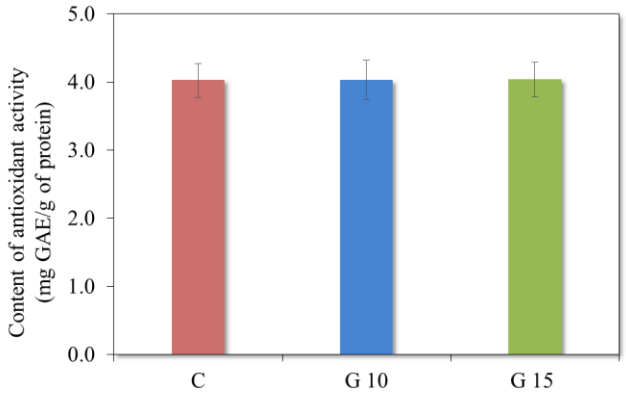

Figure 3 Evaluation of antioxidant activity using ABTS method (C - concentration 0% of garlic extract, G 10 - 10 g/kg of feed mixture, G 15 - 15 g/kg of feed mixture).

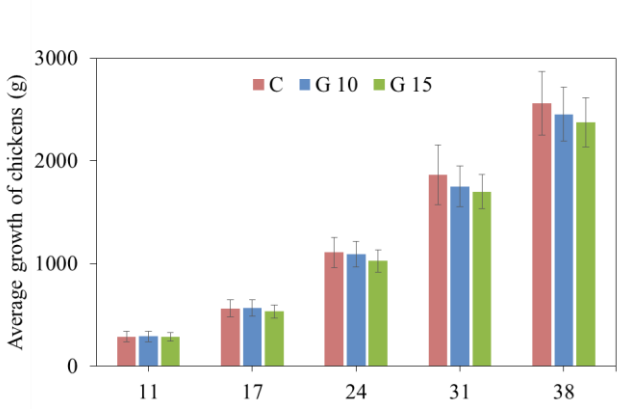

Figure 4 In the figure are shown average weekly weight gains of chickens in grams (C - concentration 0% of garlic extract, G 10 - 10 g/kg of feed mixture, G 15 - 15 g/kg of feed mixture).

obvious also at the end of the experiment (day 38) when weight of animals was higher compared to G10 (by 4%) and G15 (by 7%). Average weights of chickens of individual groups are shown in Figure 4.

Botsoglou et al. (2004) noted that well fed and healthy chickens in clean zoo hygienic conditions of adequate density do not necessarily have to positively react to growth enhancing supplements **(Botsoglou et al., 2004)**.

Differences in results of experiments by different authors may be caused by several reasons: a) difference in a used product - garlic flour, powder or garlic derivatives, b) a kind of used additive substances and concentration of active components which may differ in various experiments, c) complicated chemical composition of garlic **(Amagase et al., 2001)** and d) use of various commercial garlic preparative. Commercial garlic products may be divided according to effective substance into preparatives with raw garlic rich in allicin and preparatives with processed garlic, which contain few allicin **(Khan et al., 2012)**.

Mahmod et al. (2009) states statistically significant influence of addition of 0.5% garlic extract into feed ration on weight gain of broilers and reinforcement of feed conversion. However, influence on yield reinforcement was not proven **(Mahmood et al., 2009)**. **Stanaćev et al. (2010)** in their results state a significantly higher weight in chickens with added 2% of garlic extract into feed ration compared to control group with 0% garlic extract added. Group with added garlic extract also had lower feed conversion compared to control group **(Stanacev et al., 2011)**. According to **Onibi et al. (2009)** supplement of garlic powder in dose of 5 000 mg/kg of feed portion improved live weight, but had no influence on yield of carcasses or quality of inner organs. Adding garlic resulted in decrease in amount of abdominal fat and considerable improvement of oxidative stability of cooled chicken meat of broilers **(Onibi et al., 2009)**. Results of a study by **Raesi et al. (2010)** prove that supplementation of 1 and 3% of garlic powder had a considerable effect on live weight of chickens and improvement of feed conversion. It also had a considerable ($p < 0.001$) influence on yield of broiler carcasses **(Raeesi et al., 2010)**.

Peinado et al. (2012) proved that supplementation of feed ration with garlic derivative PTS-O (propyl propane thiosulphonate) in amount of 45 - 135 mg/kg of feed dose have a beneficial effect on decrease of number of pathogens and potentially pathogenic bacteria in gut, and on improvement of morphological structure of mucous membranes of ileum and production parameters of broiler chickens **(Peinado et al., 2012)**. The results of a study by **Adibmoradi et al. (2006)** point out that supplementation of garlic powder into feed ration is related to morphology of intestines in birds. Considerable differences compared to control group were mainly in dose of 2% of garlic in feed ration. Higher dose of garlic resulted in elongation of villi ($p < 0.05$) in duodenum, jejunum and ileum. Administration of garlic also resulted in considerable decrease in thickness of epithelium in various parts of small intestine. In all parts of small intestine, in groups being administered garlic, there was an increase ($p < 0.05$) in depth of criptae depending on dose of garlic **(Adibmoradi et al., 2006)**.

CONCLUSION

Many studies proved a potential of use of various phytogenic additives, herbs, spices or their essential oils into feed rations of poultry as alternative to antibiotics. More studies on their use are needed, considering the fact that their effect depends on many factors. Efficacy may be influenced by the amount of additive, amount of active substances, digestibility or composition of feed rations. Our expected hypothesis that garlic extract will have a beneficial effect on markers of antioxidant potential of organism and yield of observed animals was not proven. Garlic extract that was in the experiment used in doses of 10 and 15 g per 1 kg of feed ration had no considerable influence on weight gains or antioxidant activity of their organisms. It is possible that selected doses were too low and chicken organism did not react according to our expectations. For this reason in further experiments it would be advisable to increase the dose of garlic extract in chicken diets.

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